

# Neuroprotective effects of 17 $\beta$ -estradiol rely on estrogen receptor membrane initiated signals

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Besides its crucial role in many physiological events, 17 $\beta$ -estradiol (E2) exerts protective effects in the central nervous system. The E2 effects are not restricted to the brain areas related with the control of reproductive function, but rather are widespread throughout the developing and the adult brain. E2 actions are mediated through estrogen receptors (i.e., ER $\alpha$  and ER $\beta$ ) belonging to the nuclear receptor super-family. As members of the ligand-regulated transcription factor family, classically, the actions of ERs in the brain were thought to mediate only the E2 long-term transcriptional effects. However, a growing body of evidence highlighted rapid, membrane initiated E2 effects in the brain that are independent of ER transcriptional activities and are involved in E2-induced neuroprotection. The aim of this review is to focus on the rapid effects of E2 in the brain highlighting the specific role of the signaling pathway(s) of the ER $\beta$  subtype in the neuroprotective actions of E2.

**Keywords: estrogen receptor  $\alpha$ , estrogen receptor  $\beta$ , 17 $\beta$ -estradiol, neuroprotective effects, membrane initiated signals**

## INTRODUCTION

In women at approximately 51 years of age starts a profound physiological change known as menopause, which is accompanied with a dramatic decrease in estrogen levels. Taking into the account that the average of life span has increased from 50 years to over 80 years of age, an increasing number of women are living a larger portion of their lives in a chronically hypo-estrogenic state (Wise et al., 2001). The post-menopausal state of estrogen deficiency leads to different consequences throughout the female body which include the increased risk for osteoporosis and cardiovascular diseases (McEwen, 2002; Gillies and McArthur, 2010). Besides these effects, most studies have recognized that the brain is one of the organs of the body that suffer estrogen deficiency.

In addition to the well-established effects of estrogens on the reproductive behaviors and the associated brain regions (e.g., hypothalamus), 17 $\beta$ -estradiol (E2), the most active estrogen, also serves as a neurotrophic and a neuroprotective agent. Indeed, E2 influences memory formation, cognition, mood, pain sensitivity, motor coordination, neurodevelopment, and neurodegeneration (Wise et al., 2001; McEwen, 2002; Barha and Galea, 2010; Gillies and McArthur, 2010). E2 elicits either organizational effects during embryonic and neonatal development or the activational control of gene expression during the later stages of life cycle by regulating synaptic transmission, neuronal survival, neuronal, and glial differentiation (Garcia-Segura et al., 2001; Woolley, 2007; DonCarlos et al., 2009). In addition, different experimental models of neurodegeneration have shown that E2 acts as a neuroprotective factor promoting neuronal survival and tissue integrity (Maggi et al., 2004; Suzuki et al., 2006; Marin et al., 2009).

Most of these actions of E2 are mediated by estrogen receptors (i.e., ER $\alpha$  and ER $\beta$ ) belonging to the nuclear receptor super-family (Pettersson and Gustafsson, 2001; Ascenzi et al.,

2006). As members of ligand-regulated transcription factor family, classically, ERs were thought to mediate only the E2 long-term transcriptional effects. However, a growing body of evidence highlighted rapid, membrane initiated E2 effects that are independent of the ER transcriptional control (Raz et al., 2008; Arnold and Beyer, 2009; De Marinis et al., 2011; Roepke et al., 2011).

Although different subtypes of ER have been reported on neuronal membranes, emerging pharmacological and ultra-structural evidences demonstrated that ER $\alpha$  and ER $\beta$  are localized at the cell membrane where mediate rapid activation of intracellular brain signaling pathways (Vasudevan and Pfaff, 2007; Raz et al., 2008; De Marinis et al., 2010; Gillies and McArthur, 2010).

This review will highlights the rapid effects of E2 in the brain taking into account the contribution of ER $\beta$  intracellular signaling pathway in neuroprotective actions of E2.

## STRUCTURE AND ACTIVITY OF ESTROGEN RECEPTORS

Estrogen receptor alpha and ER $\beta$  (NR3A1 and NR3A2, respectively) are products of separate genes (ESR1 and ESR2, respectively) present on distinct chromosomes (locus 6q25.1 and locus 14q23-24.1, respectively; Gosden et al., 1986; Ascenzi et al., 2006; Luisi et al., 2006; Zhou et al., 2006).

Like other members of the NR family, ERs contain evolutionarily conserved structurally and functionally distinct domains. In particular, ERs are modular proteins composed of six regions named A/B, C, D, E, and F which participate in the formation of independent but interacting functional domains. DNA-binding domain (DBD or C region), the central and most conserved domain, plays a pivotal role in receptor dimerization and in the binding of specific DNA consensus sequences (i.e., estrogen responsive elements, EREs) recognized by both receptors, whereas ligand binding occurs in the C-terminal multifunctional ligand

binding domain (LBD or E region; Ascenzi et al., 2006). ERs transcriptional activation is mediated by two distinct transcription activation functions (AFs). The AF-1 is located at the N-terminus of the receptor and the AF-2 domain resides in the LBD (Ascenzi et al., 2006; Acconcia and Marino, 2011).

Classically, the ER biological activities result from modifications of the expression pattern of specific target genes. In the absence of ligands, ERs cycle on and off their DNA-binding sites (i.e., EREs). The trans-activation activity of ERs initiates after the binding of E2 which stabilizes the binding of receptors to DNA (Metivier et al., 2003; Reid et al., 2003; Picard et al., 2008; Acconcia and Marino, 2011).

In the cytoplasm of the ER-expressing cells, ER is in the monomeric state (inactive state) and forms a multiprotein complex with immunophilins and heat shock proteins (e.g., Hsp90, Hsp70, and Hsp56). Upon E2 binding, Hsps dissociate from ERs which dimerize and translocate to the nucleus. In the nucleus, the E2:ER complexes interact with ERE sequences within the promoter regions of target genes to regulate gene transcription by recruiting cofactors proteins (i.e., coactivators and corepressors) and the components of the transcription machinery (Ascenzi et al., 2006; Acconcia and Marino, 2011). Thus, the ER-macromolecular complexes facilitate the transcriptional activities of E2 responsive genes by triggering chromatin remodeling in target promoters (Ascenzi et al., 2006; Morissette et al., 2008; Acconcia and Marino, 2011).

The consensus palindromic element ERE, 5'-GGTCAnnnTG ACC-3', acts in a direction and distance independent manner, both of which are properties of an enhancer (Ascenzi et al., 2006). Nevertheless, only a fraction of known mammalian ERE displays this consensus, about one-third of the ER-regulated genes in humans do not contain ERE-like sequences (O'Lone et al., 2004; Bjornstrom and Sjoberg, 2005). Indeed, ERs can also regulate gene transcription without binding directly to DNA. This indirect genomic mechanism requires the interaction of ERs with either DNA-bound transcription factors like CREB or with Fos and Jun proteins regulating gene transcription via the activator protein 1 (AP-1) and stimulating protein 1 (Sp1; Kalaitzidis and Gilmore, 2005; Morissette et al., 2008; Acconcia and Marino, 2011).

Since 1967 it has been reported that E2 induces rapid effects in target cells that can not be related to ER transcriptional activities (Szego and Davis, 1967). Rapid effects of E2 have been reported in bone, breast, blood vessels, cancer cells, nervous system, sperm, and maturing oocytes (Nilsson and Gustafsson, 2011). E2 rapid induce the activation of several intracellular pathways (Kelly and Levin, 2001; Levin, 2005; Ascenzi et al., 2006; Acconcia and Marino, 2011). E2-induced rapid responses are important for several neural functions including cognition, behavior, stress responses, and reproduction (Farach-Carson and Davis, 2003).

E2 membrane initiated actions require ER localization at the plasma membrane. Membrane ERs are localized either in caveolae (Razandi et al., 2003) or in other membrane raft structures (Marquez et al., 2006; Acconcia and Marino, 2011). Remarkably, ER $\alpha$  and ER $\beta$  can be localized at the plasma membrane through the direct interaction with caveolin-1 to initiate the signal transduction pathway(s) (Acconcia and Marino, 2011; Roepke et al., 2011). Indeed, ERs require a post translational modification by lipid (i.e.,

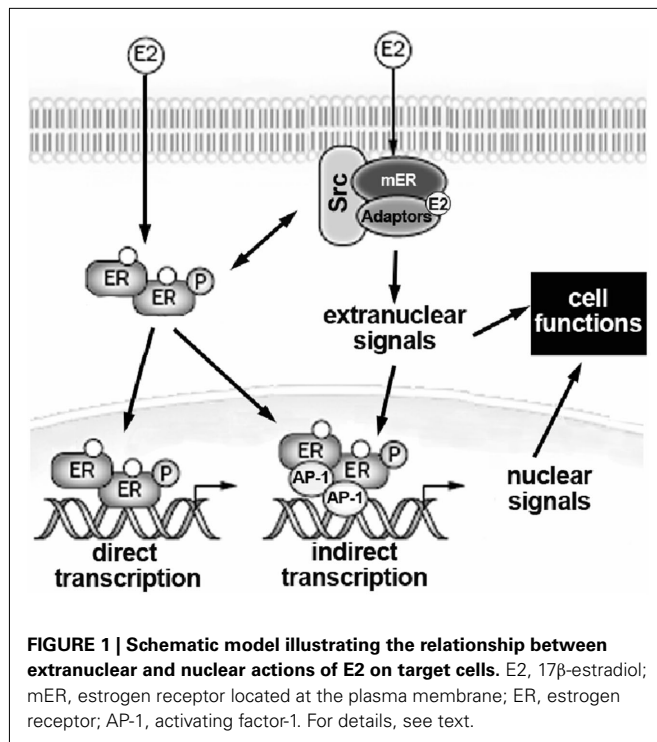
S-palmitoylation) to be localized at the plasma membrane, to interact with caveolin-1, and to initiate the E2 rapid signal cascade activation (Acconcia et al., 2005; Galluzzo et al., 2007; Pedram et al., 2007; Marino and Ascenzi, 2008).

In addition, E2 determines rapid membrane initiated effects through other receptors belonging to a protein family completely different from nuclear receptor super-family. This class of receptors includes ER-X and a G-protein coupled seven-transmembrane receptor, named GPR30 (Toran-Allerand et al., 2002; Filardo et al., 2007). ER-X is oriented in caveolar-like microdomains of postnatal, but not adult, mouse neocortical, and uterine plasma membrane. It is functionally distinct from ER $\alpha$  and ER $\beta$ , and is re-expressed in the adult brain after ischemic stroke injury, like ER $\alpha$  (Toran-Allerand et al., 2002). GPR30 is expressed in areas of the brain important for spatial learning, memory, and attention and could be an important regulator of basal forebrain cholinergic functions (Prossnitz and Barton, 2011). *In vivo* studies showed that only pharmacological concentrations of the GPR30 specific ligand G-1 (i.e., 50  $\mu$ g) could replicate the effects of the physiological concentration of E2 (i.e., 2.2  $\mu$ g) in promoting neuronal survival following global ischemia in the rodent brain (Etgen et al., 2011). Although GPR30 and its ligand may represent a new pharmacological approach for treating neuronal damage, the role of these receptors in cells from ER $\alpha$ /ER $\beta$  homozygous double knockout (DERKO) mice is not yet demonstrated; thus, at present, the possibility that GPR30 and/or ER-X mediate the E2-induced rapid transduction pathways important for brain functions is questionable.

In addition, membrane ERs activate rapid transduction pathway(s) by interacting with either cell surface receptors, such as the growth factor receptors (e.g., the EGF receptor and the IGF-1 receptor) and the metabotropic glutamate receptor, or with other signal proteins including G-proteins, non-growth factor tyrosine kinase (e.g., Src and Ras), and linker proteins (e.g., MNAR and striatin; Hammes and Levin, 2007; Gillies and McArthur, 2010). In **Figure 1** a schematic representation of nuclear and extranuclear ER activities is reported.

## ESTROGEN EFFECTS AND ACTION MECHANISMS IN THE BRAIN

E2 in the brain is either locally synthesized by the precursor testosterone or imported through the blood brain barrier from circulating factors. ERs are expressed in different brain regions such as the bed nucleus of the stria terminalis, the medial amygdala, the preoptic area, and the nucleus of the solitary tract. ER $\alpha$  is predominantly located in the hypothalamus ventromedial nucleus and in the amygdala of humans and rodents (Shughrue et al., 1998; Osterlund et al., 2000a,b,c; Gillies and McArthur, 2010). On the contrary, ER $\beta$  is the predominant form expressed in the cerebral cortex, the hippocampus, the dorsal raphe, the substantia nigra, the cerebellum, and the hypothalamic nuclei; also serotonergic and dopaminergic neurons express ER $\beta$  (Bodo and Rissman, 2006; Handa et al., 2010). In the mammalian nervous system, ER $\alpha$  and ER $\beta$  distribution patterns provide some neuroanatomical evidences for their involvement in specific brain functions. Indeed, ER $\alpha$ , but not ER $\beta$ , is crucial for E2-induced neuroreproductive functions (Ogawa et al., 1998), however many of the



non-reproductive functions of E2 in the brain can be explained only through ER $\beta$ -mediated effects (Kudwa et al., 2006; Antal et al., 2008).

Although E2 is the predominant circulating sex steroid hormone after puberty in females, it also plays a pivotal role in the male brain, E2 being synthesized from steroid precursors (e.g., testosterone by P450 aromatase enzyme). Testosterone produced during the critical developmental window in male is a key factor in the masculinization/defeminization process (Huhtaniemi, 1994). Morphological, cellular, and molecular differences exist in diverse male and female brain regions important for cognition and memory (e.g., the hippocampus, the amygdala, the cortex, and the regions controlling sensorimotor and reward systems; see Gillies and McArthur, 2010).

Functional differences in male and female brain explain the diverse responses to environmental challenges and different vulnerabilities to behavioral and neurological disorders. Striking differences between sexes have been reported concerning the symptoms, the prevalence, the progression, and the severity of several neurodegenerative diseases. Indeed, pre-menopausal women seem to be less prone to Alzheimer's, Parkinson's, and Huntington's diseases than males or post-menopausal women (Amantea et al., 2005; Morissette et al., 2008 and literature cited therein). Moreover, the pivotal role of E2 on higher brain functions including mood, anxiety, fear, learning, and memory have been confirmed by epidemiological data which indicate that pre-menopausal women seem to be more vulnerable than men to develop anxiety or depression behavior (Schneier et al., 1992; Kessler et al., 1994; Breslau et al., 1995; Seeman, 1997; Luine, 2008; Watson et al., 2010).

## E2 AS A NEUROTROPHIC FACTOR

E2 exerts multiple and diverse actions in the brain throughout the life span from development to senescence. E2 exerts neurotrophic and neuroprotective effect in adult brain regulating the synthesis and the secretory patterns of neurotransmitters, neuropeptides, and their receptors, influencing the sexual behavior as well as gonadotropin and prolactin secretion (Pfaff et al., 1994). Moreover, as it does during development.

E2 is a potent trophic factor that influences brain development and differentiation, plasticity and cell survival both during fetal life and in the early postnatal period. These trophic effects include the modulation of cell migration, neuronal growth, formation, and elimination of synapses, and neurogenesis (Pfaff et al., 1994; Garcia-Segura et al., 1996; Wise et al., 2001; Gillies and McArthur, 2010).

In nearly all mammalian species, including humans, adult neurogenesis has been observed in two brain regions characterized by neuronal progenitor cells (NPCs): the dentate gyrus of the hippocampus and the lateral walls of lateral ventricles (Gould et al., 1997, 2001; Eriksson et al., 1998; Amrein et al., 2004; Handa et al., 2010). E2 can promote the proliferation of hippocampal neuron progenitor cells *in vitro* and neurogenesis in rat hippocampus *in vivo*, both under physiological and pathological conditions (Tanapat et al., 1999, 2005; Brannvall et al., 2002, 2005; Suzuki et al., 2007). In human NPCs, E2-induced proliferation seems to be mediated only by ER $\beta$  (Wang et al., 2008), which represent the predominant ER subtype in these cells (Fried et al., 2004; Hong et al., 2004). Interestingly, both E2 and the ER $\beta$  selective-ligand diarylpropionitrile (DPN) are able to induce *in vitro* human NPCs proliferation through the activation of ER $\beta$  that in turn leads to a rapid increase in kinase levels (Squires et al., 2002; Ussar and Voss, 2004; Hata et al., 2005; Chambard et al., 2007; Wang et al., 2008; Handa et al., 2010).

Neurogenesis is involved in hippocampus-dependent learning and memory, it is highly correlated with the chronic antidepressant treatment of male and female rodents affected by the depressive-like behavior; the antidepressant treatment increases also cell proliferation (Malberg and Duman, 2003; Vollmayr et al., 2003; Green and Galea, 2008). Moreover, neurogenesis could represent a neuroprotective action of E2 in neurodegenerative conditions such as Alzheimer's disease, which is accompanied by a decline of neurogenesis (Wang et al., 2007, 2010; Rodriguez et al., 2008; Demars et al., 2010). In addition to the reported effects, E2 in the hippocampus enhances learning and memory promoting the formation of new dendritic spines and new excitatory synapses, increasing the expression of NMDA receptors (Adams et al., 2004), and enhancing long-term potentiation (LTP; Smith and McMahon, 2006; Liu et al., 2008).

Dramatic changes in E2 levels, due to surgery and/or to menopause, are associated with changes in incidence and symptomatology of anxiety and depression, in memory ability, and performance in visual-spatial tasks (Hampson and Kimura, 1988; Phillips and Sherwin, 1992). E2 replacement can improve mood score (Wise et al., 2001; Walf and Frye, 2006) and counteract memory loss in both verbal and non-verbal memory test and attention (Campbell and Whitehead, 1977; Halbreich, 1997; Rhodes and Frye, 2004; Sherwin, 2005). Further confirmation of the

E2 involvement in higher brain functions derives from animal models. In fact, an increased anxiety-related behavior has been observed in both rats and mice as a consequence of E2 decline levels (Gupta et al., 2001; Morgan and Pfaff, 2001, 2002; Bodo and Rissman, 2006; Inagaki et al., 2010; Jacome et al., 2010).

### MECHANISMS UNDERLYING E2-INDUCED EFFECTS ON NEUROTROPHISM

The hippocampus seems to represent the main brain area that mediate E2 effects on the affective behavior and the cognitive function. Together with the amygdala area, hippocampus is considered an important component of the limbic system and a regulator of the hypothalamic–pituitary–adrenal axis (HPA; Walf and Frye, 2006). Although the proper mechanism by which E2 acts in hippocampus is openly debated, growing evidence indicates that E2 effects are rapid and could require a membrane-associated receptor (Walf and Frye, 2007, 2008).

Although rapid membrane-mediated effects of E2 have been identified in the hippocampus and in some hypothalamic nuclei (Ronnekleiv and Kelly, 2005; Roepke et al., 2009), they may occur throughout the whole central nervous system. Membrane initiated E2 signaling involves the rapid activation of various protein kinase cascades, including protein kinase C (PKC), protein kinase A (PKA), phosphatidylinositol-3 kinase (PI3K), protein kinase B (or Akt), and mitogen-activated protein kinase (MAPK), which in turn modulate protein phosphorylation, cation channel activity, and gene expression (Roepke et al., 2011). Furthermore, E2-activated signaling pathways modulate the intracellular  $\text{Ca}^{2+}$  levels regulating the activation of protein kinases including the  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase II (CaMKII; Gillies and McArthur, 2010).

A rapid induction of the cAMP/PKA pathway, mediating the effect of E2 on the change of  $\text{K}^+$  fluxes in GnRH neurons (Gu and Moss, 1996; Malyala et al., 2005), has been demonstrated in hippocampal neurons (Chen et al., 1998; Ascenzi et al., 2006). Moreover, E2 can attenuate the ability of  $\mu$ -opioid and GABA receptor-mediated activation of G-protein-gated inwardly rectifying  $\text{K}^+$  channels (Kelly et al., 1992). Notably, PKA is involved in this E2-induced pathway, in fact forskolin, a PKA activator, and cAMP can mimic the E2 effects (Lagrange et al., 1997). Several *in vitro* reports, using hippocampal primary cell culture, have demonstrated that E2 can activate intracellular signaling pathway(s) to indirectly affect the genomic activity through transcriptional regulators such as the cAMP response element binding protein (CREBP; Kelly and Levin, 2001; Wade and Dorsa, 2003; Lee et al., 2004). Notably, CREBP is regulated through phosphorylation by several signaling kinases, including Akt, CaMKII, and MAPK (Brunet et al., 2001; Sawai et al., 2002). The E2-dependent phosphorylation of CREBP has been shown to be a critical step in neuronal survival, synaptic plasticity, and learning as well as particularly in processes that lead to the “generation” of new dendritic spines in dissociated hippocampal neurons (Murphy and Segal, 1996, 1997).

The regulation of AMPA receptors is a key mechanism for the potentiation of synaptic strength in LTP, which is considered a cellular model of learning and memory (Bliss and Collingridge, 1993; Malinow and Malenka, 2002; Malenka and Bear, 2004). Treatment

of hippocampal slices with the ER $\beta$  agonist WAY-200070 induces a PKA-dependent phosphorylation of the GLUR1 subunit, which leads to the increased expression of AMPAR at the cell surface. Furthermore, ER $\beta$  activation induces morphological changes in hippocampal neurons *in vivo*, including increased branching and density of spines (Liu et al., 2008).

### E2 AS A NEUROPROTECTIVE AGENT

E2 plays a significant neuroprotective role in the brain, this explains its ability to ameliorate symptoms and to decrease the risk of neurodegenerative events and of ischemic stroke, Alzheimer's, Parkinson's, and Huntington's diseases (Amantea et al., 2005). In particular, E2 displays neuroprotection against A $\beta$  amyloid toxicity (Fitzpatrick et al., 2002; Guerra et al., 2004) and oxidative stress-induced neuronal death (Bae et al., 2000; Wang et al., 2006; De Marinis et al., 2010) in many neuronal cell lines and tissue preparations. Moreover, E2 seems to protect neurons against apoptosis by reducing reactive oxygen species (ROS) production (Razmara et al., 2007; Irwin et al., 2008; Numakawa et al., 2011), inhibiting the neurotoxic effect of oxidized LDL and glutamate (Bhavnani, 2003), maintaining the  $\text{Ca}^{2+}$  homeostasis (Ba et al., 2004a), regulating pro-apoptotic caspase activities (Bao et al., 2011), and maintaining mitochondrial membrane integrity (Simpkins and Dykens, 2008; Arnold and Beyer, 2009; Wang et al., 2011). Furthermore, E2 displays neuroprotection against ischemic injuries (Dubal et al., 2001; Carswell et al., 2004). Remarkably, the inhibition of cell death by E2 has been demonstrated in neuronal cell lines, such as NT2 neurons, PC12, mouse hippocampal T22 neurons, as well as mouse and human neuroblastoma cells exposed to glutamate, hydrogen peroxide, and AMPA (Zaulyanov et al., 1999; Ba et al., 2004b; Sribnick et al., 2004; Amantea et al., 2005; De Marinis et al., 2010).

Both nuclear and membrane initiated mechanisms of action are required for the neuroprotective role of E2. ERs can interact directly with the MAPK pathway resulting in the activation of a wide variety of transcription factors involved in neuronal survival (Morissette et al., 2008). Notably, MAPK signaling is accompanied by the activation of CREBP, the induction of Bcl-2 expression, and the inhibition of the apoptotic cascade (Azcoitia et al., 2011). In particular, the E2 neuroprotective effects against the quinolinic acid-induced toxicity in rat hippocampus cells is abolished by pre-treatment with U0126, an inhibitor of MAPK/ERK kinase (Kuroki et al., 2001).

Estrogen receptors can also interact with the PI3K signaling pathway leading to activation of the effector protein kinase Akt. Activated Akt, in turn, can modulate the expression of apoptosis inhibitors (i.e., Bcl-2 and Bcl-x) or inducers (i.e., Bax and Bad), thus influencing cell death. Similarly, phosphorylation of either caspase-9 or the fork head transcription factors by Akt could further block the apoptosis induction in neurons. Therefore, Akt promotes cell survival opposing to the apoptosis by a variety of routes. In particular, the PI3K/Akt pathway is shown to be a critical survival mechanism in the neuroprotective activities of E2 in dopamine neurotransmission in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice model of Parkinson's disease. In this model, Akt activates anti-apoptotic proteins and inactivates glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) activity by increasing

the phosphorylation level of Ser9 and Ser21. GSK3 $\beta$  is highly expressed in the central nervous system; the activation of GSK3 $\beta$  facilitates neuronal death whereas its inactivation promotes cellular mechanisms involved in neuronal survival pathways. Furthermore, evidence shows that GSK3 $\beta$  could play a key role in the pathogenesis of the Alzheimer's disease (Morissette et al., 2008).

E2 rapidly promotes the phosphorylation and the subsequent inactivation of the voltage dependent anion channel (VDAC), a mitochondrial porin also found at the neuronal membrane, where it appears to be involved in redox regulation, extrinsic pathway, and A $\beta$  amyloid neurotoxicity. This E2 effect requires the activation of PKA and Src-kinase which may be relevant to maintain this channel inactivated (Herrera et al., 2011).

### ER $\beta$ -SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN E2-INDUCED NEUROPROTECTION

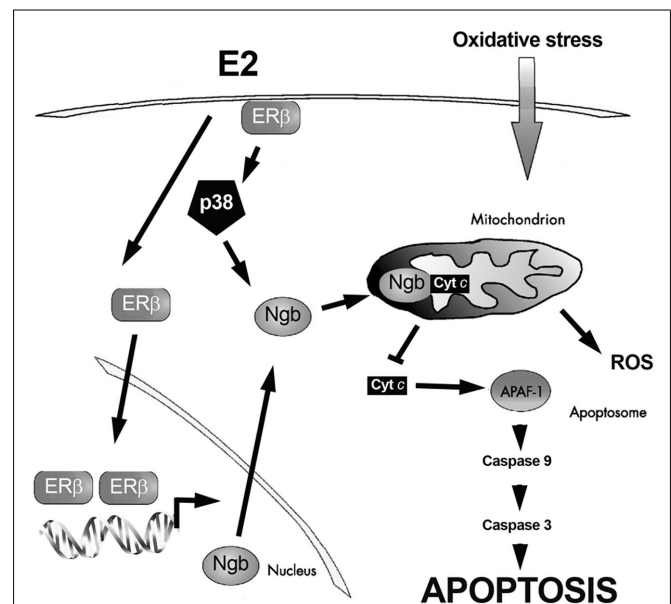
Whereas ER $\alpha$  is a critical link in the mechanism of E2 neuroprotection, the role of ER $\beta$  is less clear. By now there is growing evidence from *in vitro* and *in vivo* studies which shows the involvement of ER $\beta$ -mediated rapid signaling in neuroprotection. Despite both ERs are expressed by hippocampal neurons, E2 effects on hippocampus seem to be mediated by ER $\beta$  which is more prevalent than ER $\alpha$  (Shughrue et al., 1996, 1998; Laflamme et al., 1998; Hileman et al., 1999; Mitra et al., 2003). Different studies, based on knockout animal models, have been performed to determine whether ER $\beta$  in the hippocampus is a E2 target. Notably, E2-treated wild-type mice exhibit a less depressive-like behavior than controls, this E2 effect is lost in ER $\beta$  knockout mice  $\beta$ ERKO (Rocha et al., 2005; Weiser et al., 2008). Furthermore, ovariectomized female rats treated with the ER $\beta$  agonist DPN show a decrease of the anxiety-type behavior (Weiser et al., 2009) and the administration of ER $\beta$ -selective ligands directly in the hippocampus decreases the depressive-like behavior suggesting the pivotal role of this brain area (Walf and Frye, 2007). All together, these results indicate that the ER $\beta$ -mediated mechanism(s) is pivotal for the E2-induced synaptic plasticity modulation in the hippocampus and, ultimately, for E2-induced learning and memory.

Exposure of human SK-N-BE neuroblastoma cell to H<sub>2</sub>O<sub>2</sub> (=50  $\mu$ M) induces the increase of ROS in cells (followed by oxidation of proteins, lipids, and DNA), glutathione depletion, mitochondria dysfunction, intracellular Ca<sup>2+</sup> increase, and caspase-3 activation followed by apoptotic cell death (Wang et al., 2006). Recently, cells pre-treatment with physiological E2 concentration has been demonstrated to decrease cell death and to reduce the activation of the pro-apoptotic cascade (i.e., caspase-3 activation and PARP cleavage; De Marinis et al., 2010).

Although SK-N-BE cells express different level of both ER subtypes, the E2-protective effects against H<sub>2</sub>O<sub>2</sub>-induced neuron toxicity required rapid ER $\beta$ -activities (De Marinis et al., 2010). In fact, cell pre-treatment with the specific ER $\beta$ -inhibitor THC completely prevents E2 effects (De Marinis et al., 2010). Interestingly, the monomeric globin neuroglobin (Ngb), displaying a protective function in the brain (Fago et al., 2008; Burmester and Hankeln, 2009; Yu et al., 2009; De Marinis et al., 2010, 2011; DellaValle et al., 2010), is pivotal in ER $\beta$ -mediated effects on neuroprotection (De Marinis et al., 2010). The E2 effect on Ngb expression is rapid (1 h), persistent (24 h), specific, and mediated by the ER $\beta$

subtype. In particular, the E2-induction of Ngb increase requires the ER $\beta$ -mediated rapid activation of p38/MAPK. Moreover, cell pre-treatment with the transcription inhibitor actinomycin and the translation inhibitor cycloheximide completely prevents the increase of E2-induced Ngb levels. Note that the integration between rapid and genomic ER $\beta$ -mediated events is required to guarantee rapid and persistent E2 effects in neuroprotection against H<sub>2</sub>O<sub>2</sub> toxicity (De Marinis et al., 2010). Furthermore, in mouse primary cortical astrocytes, one of the cellular targets of E2 in the brain (Arevalo et al., 2010), E2 stimulation reduces lipopolysaccharide-induced cytokine production only in the presence of ER $\beta$ -induced increase of Ngb protein levels (Marino M., unpublished data). As a whole, these data suggest a new membrane initiated signaling pathway involving ER $\beta$ , p38/MAPK, and Ngb in E2-induced neuroprotection (Figure 2).

However, conflicting data are available on the role played by ER $\beta$  in neuroprotection. Several *in vivo* studies in ER $\alpha$  ( $\alpha$ ERKO) and ER $\beta$  ( $\beta$ ERKO) knockout mice model of cerebral ischemia report the pivotal role of ER $\alpha$ , but not of ER $\beta$ , in the mechanism(s) associated with the estrogen-mediated neuroprotection in different brain regions, such as the cortex and the striatum (Dubal et al., 2001). Moreover, ER $\beta$  seems to be not involved in E2-induced neuroprotection against the A $\beta$  amyloid toxicity in septal-derived SN56 cells and in hippocampal-derived cell line HT22 (Guerra et al., 2004; Marin et al., 2007). On the other hand, a pronounced



**FIGURE 2 | Schematic model illustrating the ER $\beta$ -dependent**

**E2-induced neuroprotection against oxidative stress.** Exposure to oxidative stress induces cytochrome c (cyt c) release into the cytoplasm. Once in the cytosol cyt c mediates the activation of the adaptor molecule apoptosis–protease activating factor-1 (Apaf-1), generating the apoptosome. Apoptosome can recruit caspase-9 favoring proteinase activation. These events induce the catalytic maturation of caspase-3, which mediates the biochemical and morphological features of apoptosis. E2, via the synergy between extranuclear and nuclear ER $\beta$  activities, increases neuroglobin (Ngb) levels, reallocates Ngb at mitochondria, facilitates Ngb-cytochrome c interaction, and prevents apoptosome formation. For details, see text.



E2 neuroprotection against  $\beta$ -amyloid peptide in HT22 cells stably transfected with ER $\beta$  (HTER $\beta$ ) and with ER $\alpha$  (HTER $\alpha$ ) has been reported. In particular, E2 induced a similar time course of MAPK activation in ER $\alpha$  or ER $\beta$  expressing cells (maximal activation at 15 min) which was necessary for neuroprotection against  $\beta$ -amyloid toxicity (Fitzpatrick et al., 2002). Moreover, the loss of ER $\alpha$  did not enhance tissue damage in the female mice after experimental stroke (Sampei et al., 2000). Furthermore, Carswell et al. (2004) showed that administration of the specific ER $\beta$  agonist DPN reduced ischemic damage in the striatum and in the CA1 region of the hippocampus in ovariectomized C57B1/6J mice while pre-treatment with the ER $\alpha$  agonist PPT had no effect on the extension of the area affected by neural damage.

Further evidence for the involvement of ER $\beta$ -mediated effect(s) in neuroprotection derives from studies on the Mongolian Gerbil global cerebral ischemia model. In this *in vivo* model, it has been demonstrated that the plant-derived phytoestrogen genistein, a mild-selective agonist for ER $\beta$  (with more than sevenfold higher binding affinity for ER $\beta$  than ER $\alpha$ ; Kuiper et al., 1997), reversed ischemic-induced memory impairment, evaluated through both behavior and object recognition tests, and fully promoted survival of pyramidal cells in the CA1 hippocampal subfield. Moreover, the use of the selective ER $\beta$  antagonist PHTTP completely prevented the neuroprotective effect(s) of genistein (Donzelli et al., 2010). These findings confirmed data obtained administering genistein before injury in focal cerebral ischemia in the mouse (Trieu et al., 1999), and in global cerebral ischemia in rats (Schreihofer et al., 2005) and gerbils (Kindy, 1993).

## CONCLUSION

In mammals, E2 exerts a profound influence on multiple brain circuits involved in reproductive and non-reproductive physiology and behavior. In fact, E2 modulates inflammatory processes, anxiety, depressive-like behaviors, and cognitive functions. In addition, an increasing number of evidence suggests that E2 exerts significant neuroprotective effects against a variety of neurodegenerative pathologies such as the Alzheimer's and Parkinson's diseases.

Several studies based on both *in vitro* and *in vivo* models demonstrated that E2 can exert its neuroprotective role against multiple types of insults including A $\beta$ -amyloid toxicity, ischemic

injuries, and other oxidative stresses. Furthermore, E2 acts in the brain modulating synaptogenesis, neurogenesis, inflammation, and protecting neurons against apoptosis.

In addition to the well-established ER $\alpha$  and ER $\beta$  direct transcriptional activities, both ER subtypes are also involved in rapid, extranuclear, E2 actions in the brain. Membrane initiated estrogen signaling involves the rapid activation of different protein kinase pathways, including PKC/PKA/MAPK and PI3K/Akt, to modulate signal transduction, protein phosphorylation, and gene expression suitable for cell survival.

Although the specific role of ER $\alpha$  and ER $\beta$  in neuroprotection is openly debated, the studies reviewed here clearly support the idea that E2 acts as neuroprotective factor through direct and indirect genomic mechanisms that converge with rapid membrane initiated effects on signaling proteins in order to regulate cell death and survival factors.

The field of E2-induced neuroprotection is booming in recent years. For many years ER $\alpha$  has been considered a critical link in the mechanism of protection of cell death, but the increasing amount of data propose that also ER $\beta$  could play a pivotal role in neuroprotection through the integration between the direct genomic and membrane initiated activities. Thus, a profound knowledge of the membrane ER $\beta$  initiated pathways is required in order to identify critical players of these mechanisms that might represent target candidates for therapeutic development in the treatment of post-menopausal neuronal deficiency and neurodegenerative diseases. Further studies are needed to better characterize the modulation and the modes of action of Nrg both in *in vitro* and *in vivo* models in order to provide a rationale for its pharmacological exploitation as an effective neuroprotective mediator of E2-dependent actions in the brain.

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